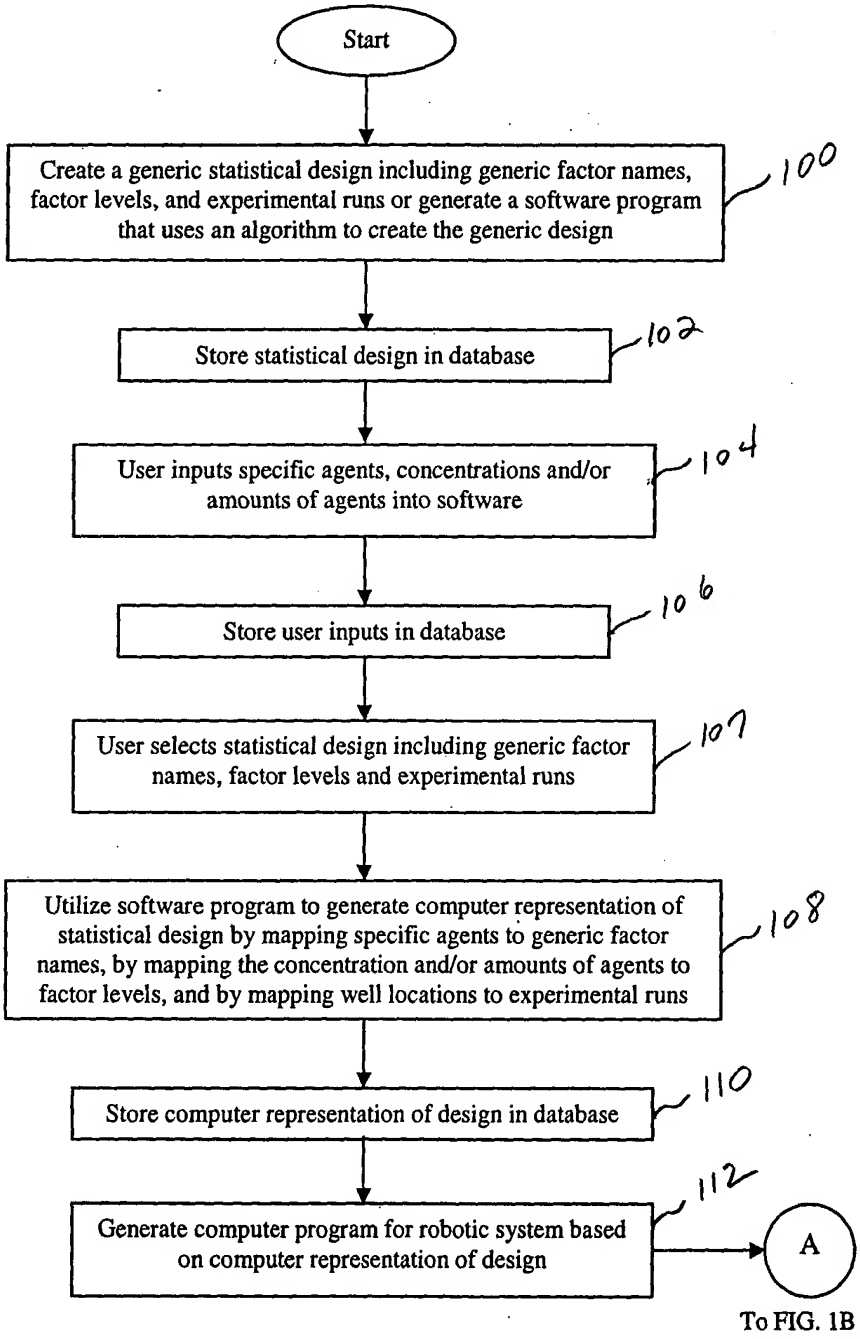

Start
Create a generic statistical design including generic factor names, factor levels, and experimental runs or generate a software program that uses an algorithm to create the generic design
100
Store statistical design in database
102
User inputs specific agents, concentrations and/or amounts of agents into software
104
Store user inputs in database
106
User selects statistical design including generic factor names, factor levels and experimental runs
107
Utilize software program to generate computer representation of statistical design by mapping specific agents to generic factor names, by mapping the concentration and/or amounts of agents to factor levels, and by mapping well locations to experimental runs
108
Store computer representation of design in database
110
Generate computer program for robotic system based on computer representation of design
112
A
To FIG. 1B

FIG 1A

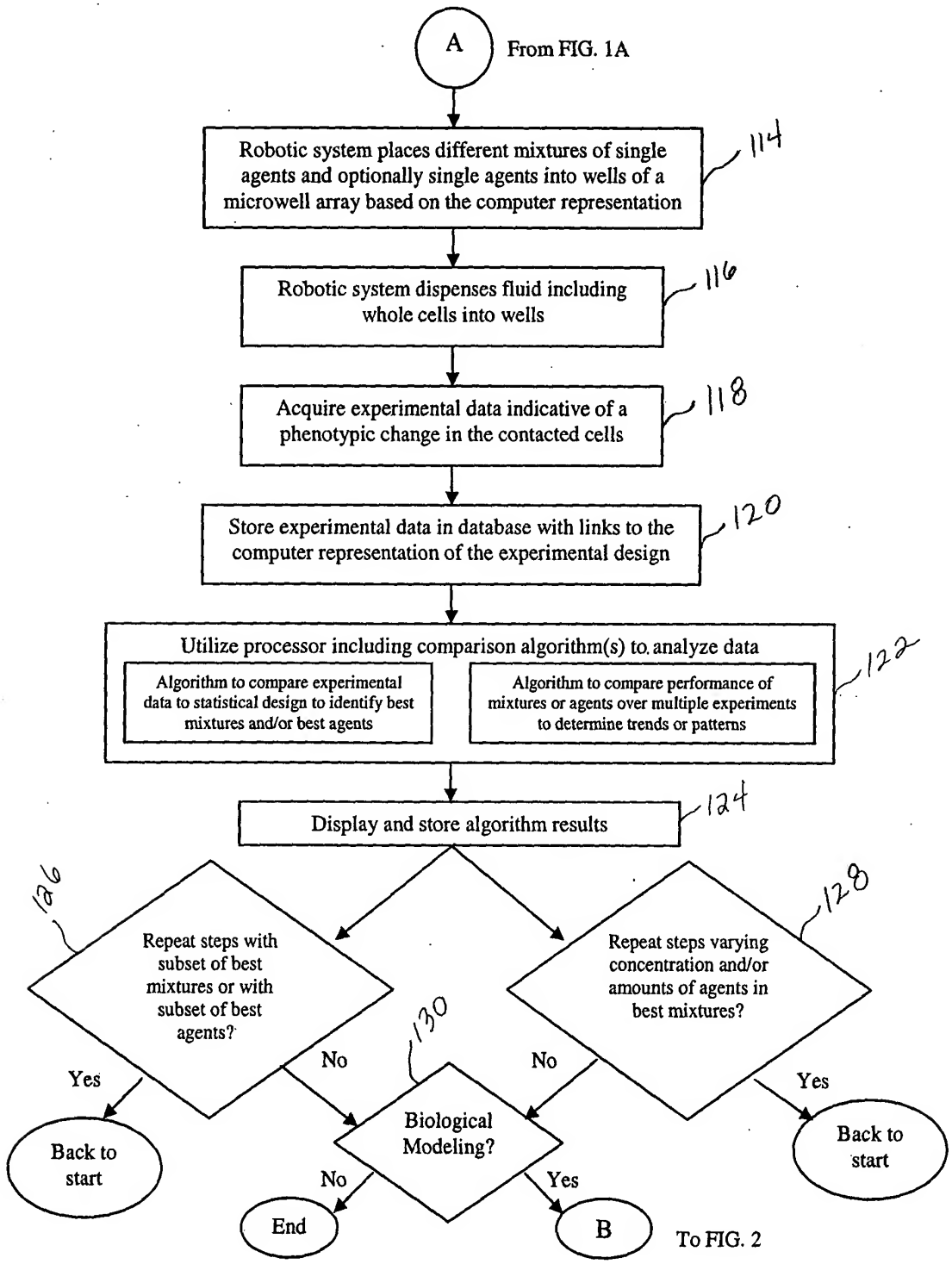

A
From FIG. 1A
Robotic system places different mixtures of single agents and optionally single agents into wells of a microwell array based on the computer representation
114
Robotic system dispenses fluid including whole cells into wells
116
Acquire experimental data indicative of a phenotypic change in the contacted cells
118
Store experimental data in database with links to the computer representation of the experimental design
120
Utilize processor including comparison algorithm(s) to analyze data
Algorithm to compare experimental data to statistical design to identify best mixtures and/or best agents
Algorithm to compare performance of mixtures or agents over multiple experiments to determine trends or patterns
122
Display and store algorithm results
124
126
Repeat steps with subset of best mixtures or with subset of best agents?
Yes
Back to start
No
130
Biological Modeling?
No
End
Yes
B
To FIG. 2
128
Repeat steps varying concentration and/or amounts of agents in best mixtures?
No
Yes
Back to start

FIG 1B

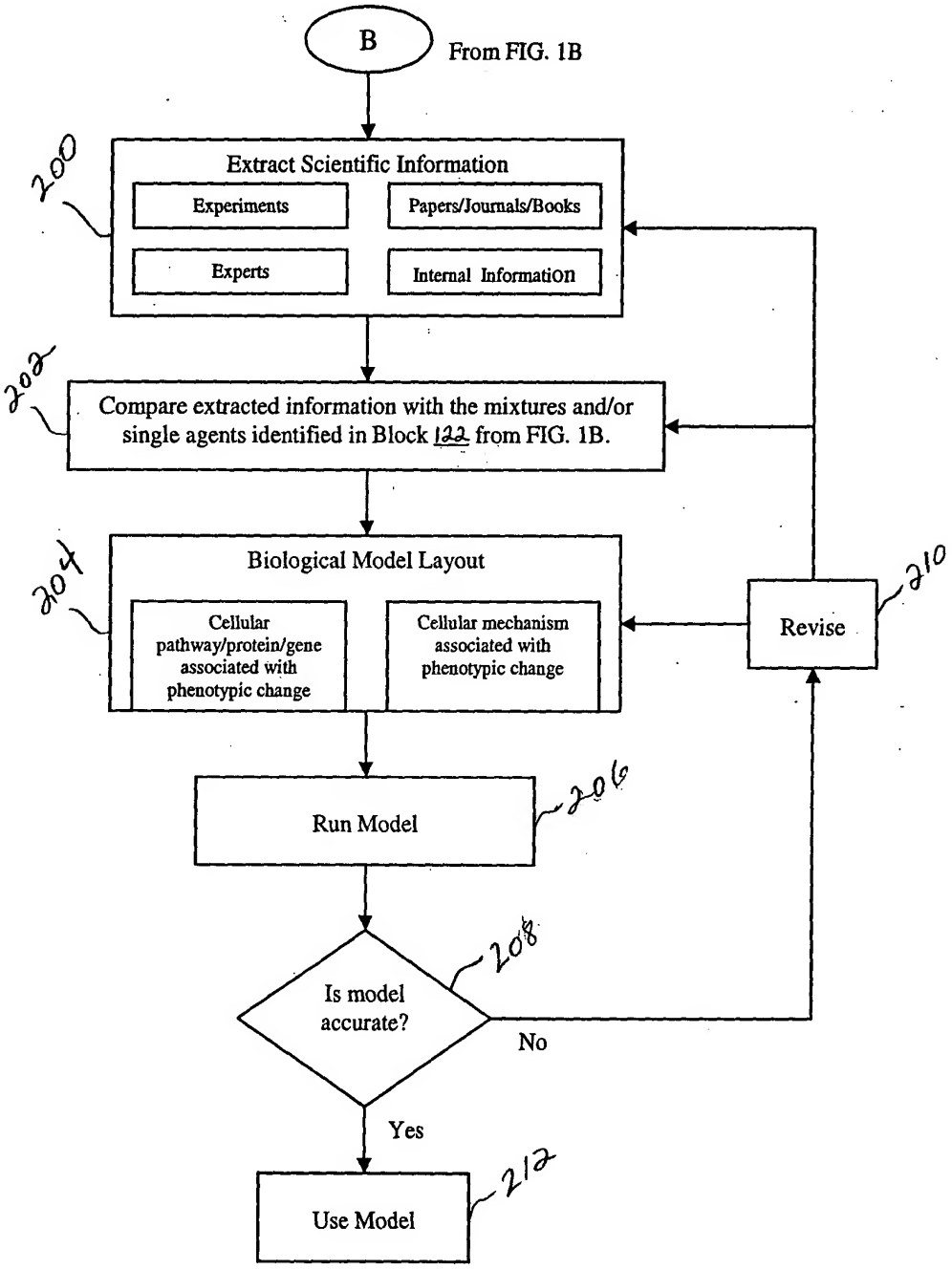

B
From FIG. 1B
200
Extract Scientific Information
Experiments
Papers/Journals/Books
Experts
Internal Information
202
Compare extracted information with the mixtures and/or single agents identified in Block 122 from FIG. 1B.
204
Biological Model Layout
Cellular pathway/protein/gene associated with phenotypic change
Cellular mechanism associated with phenotypic change
210
Revise
206
Run Model
208
Is model accurate?
No
Yes
212
Use Model

FIG 2

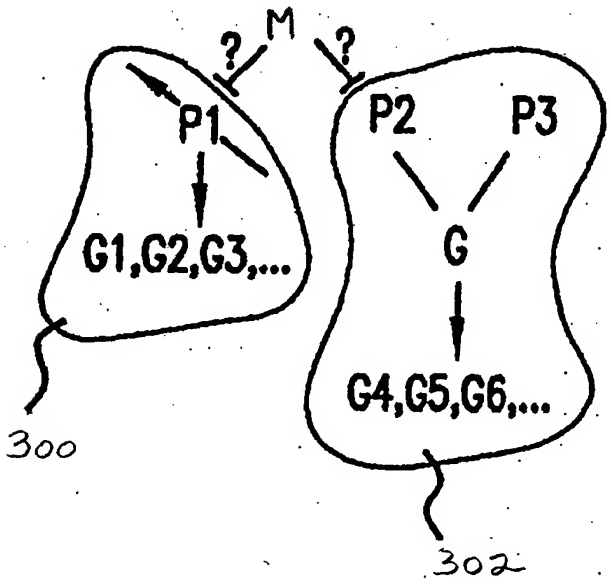


FIG. 3

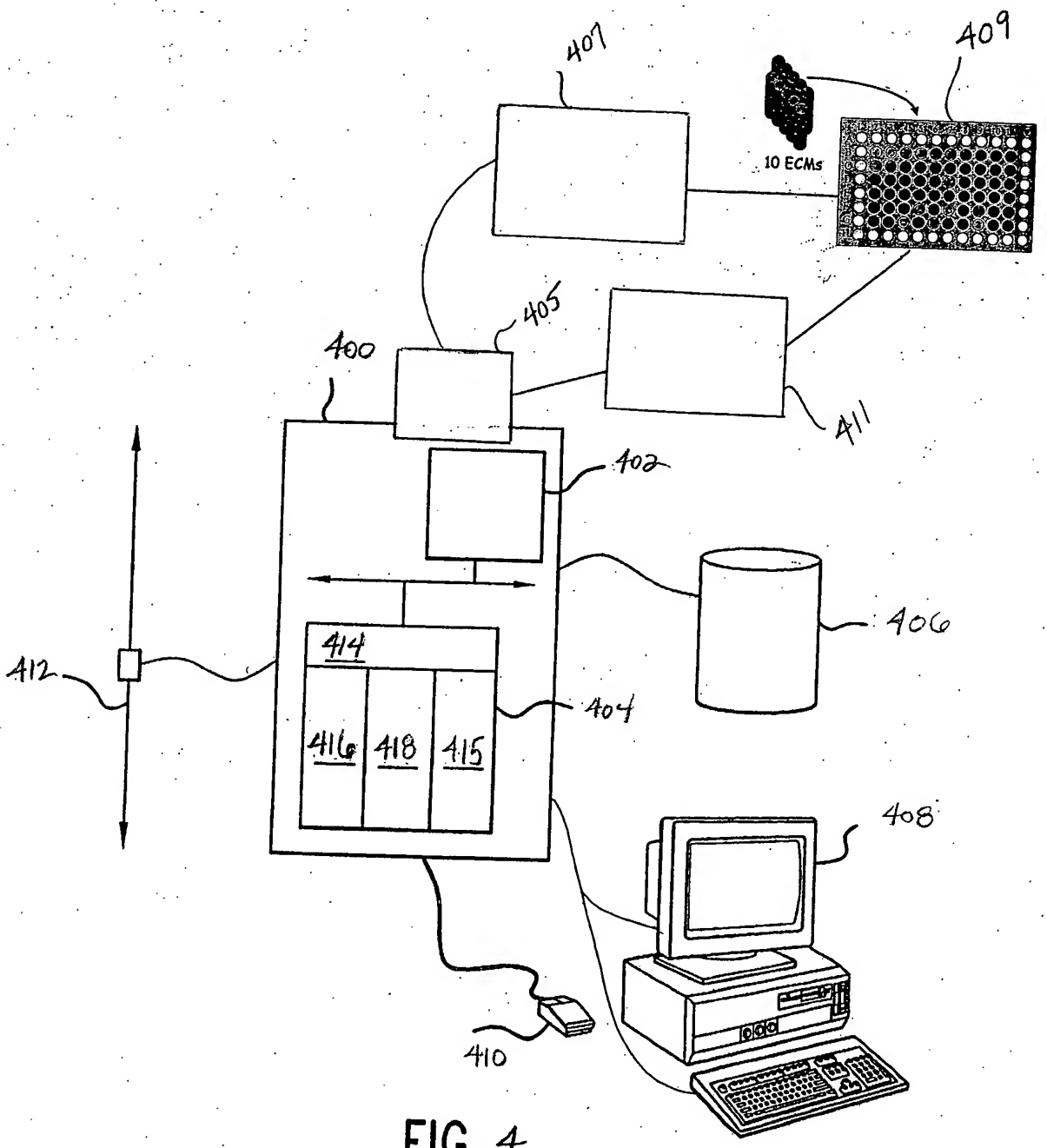

407
409
10 ECMs
405
400
411
402
406
414
412
404
416
418
415
408
410

FIG. 4

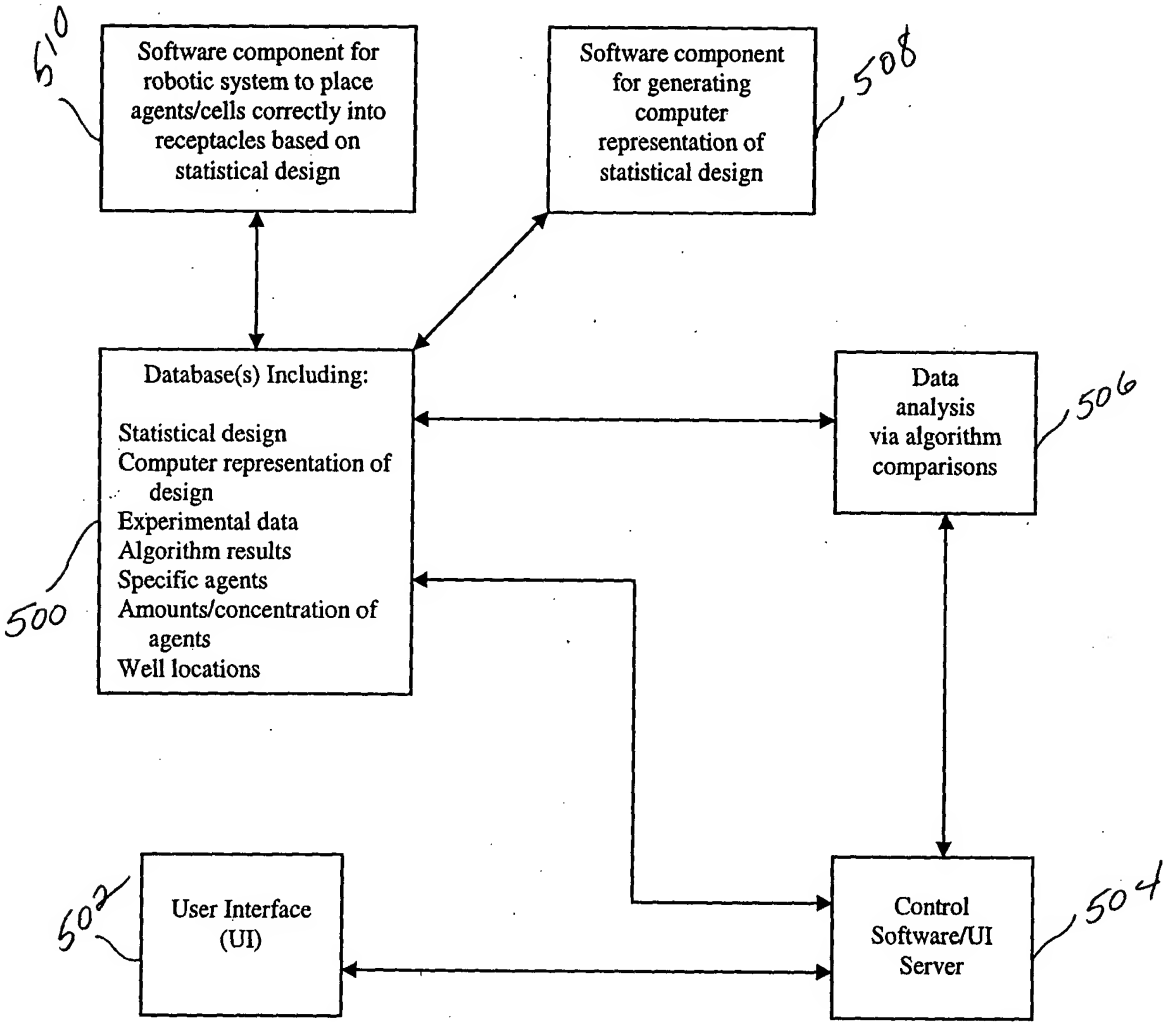

510
Software component for robotic system to place agents/cells correctly into receptacles based on statistical design
Software component for generating computer representation of statistical design
508
Database(s) Including:
Statistical design
Computer representation of design
Experimental data
Algorithm results
Specific agents
Amounts/concentration of agents
Well locations
500
Data analysis via algorithm comparisons
506
User Interface (UI)
502
Control Software/UI Server
504

FIG 5

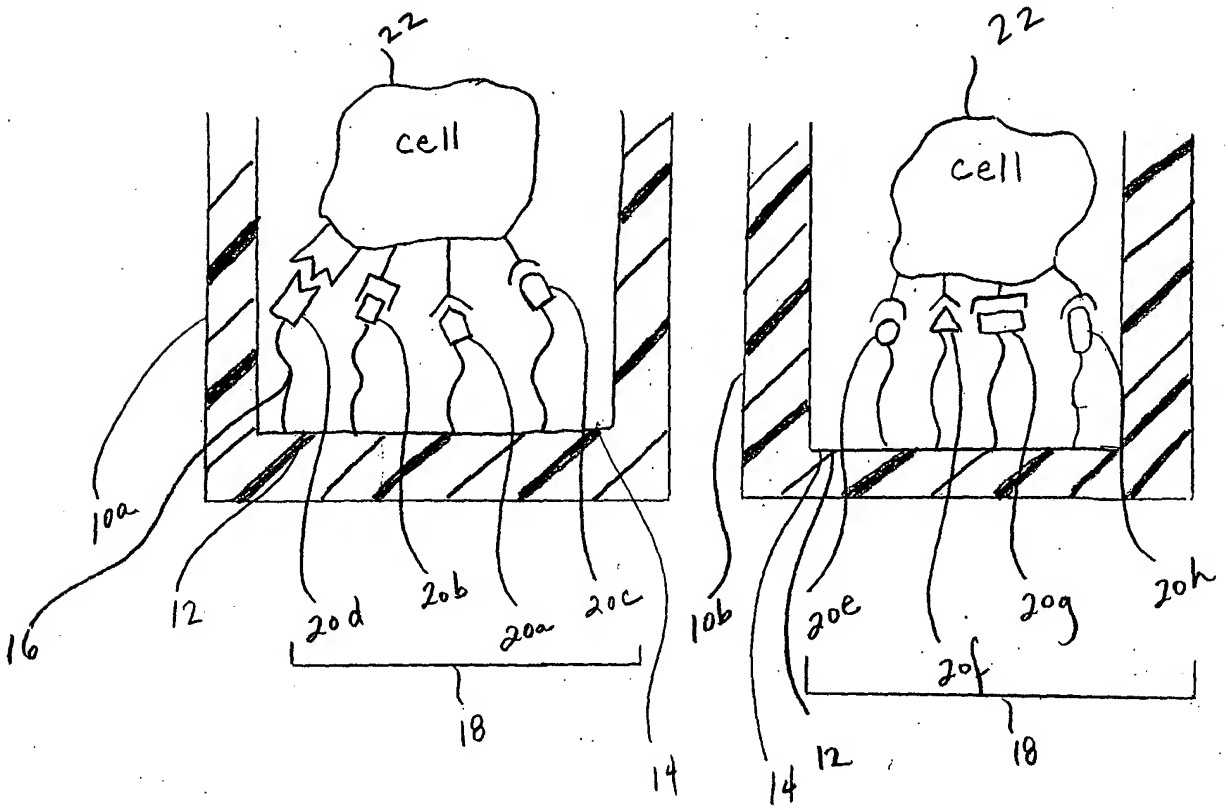

22
cell
10a
16
12
20d
20b
20a
20c
18
14
22
cell
10b
14
12
20e
20g
20h
20f
18

Fig. 6

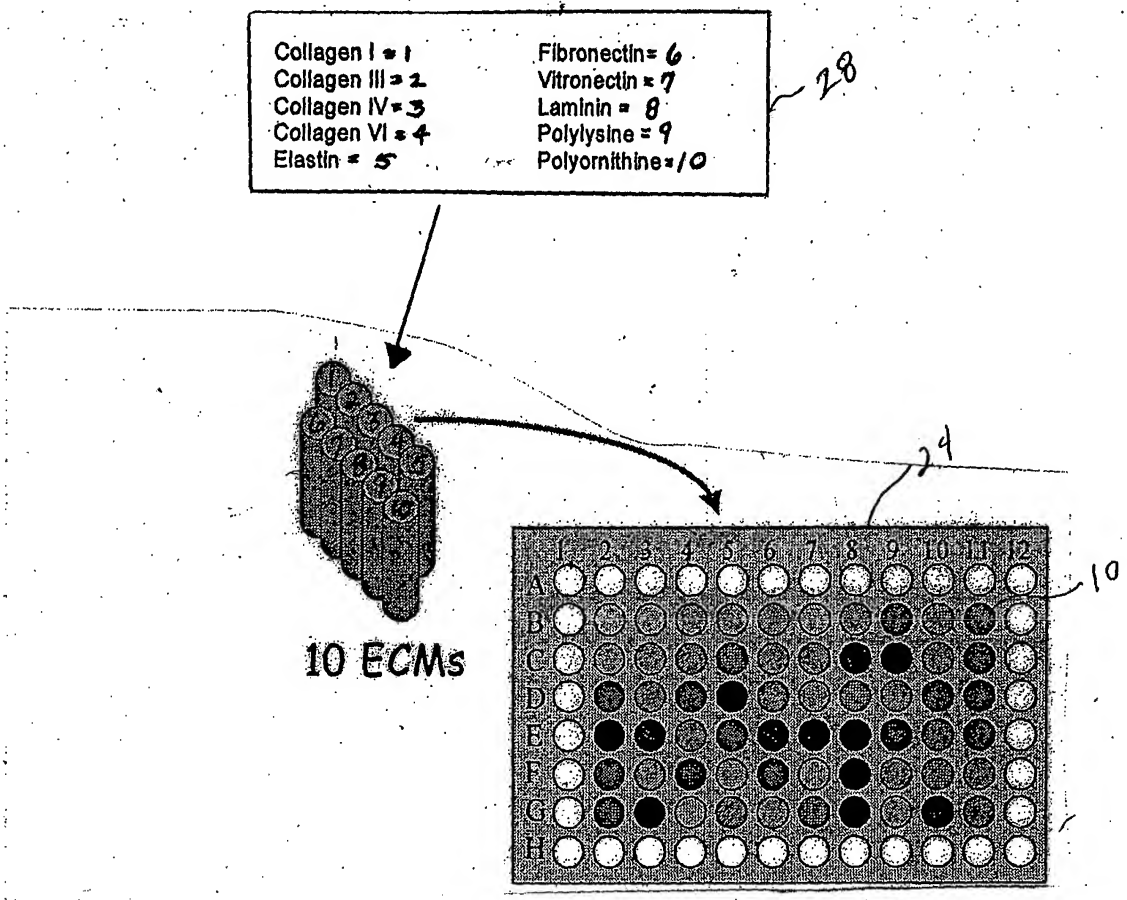
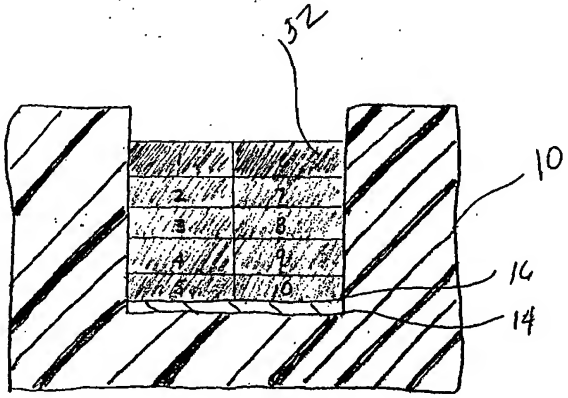

Collagen I = 1
Collagen III = 2
Collagen IV = 3
Collagen VI = 4
Elastin = 5
Fibronectin = 6
Vitronectin = 7
Laminin = 8
Polylysine = 9
Polyornithine = 10
28
10 ECMs
24
10
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H

Fig. 7

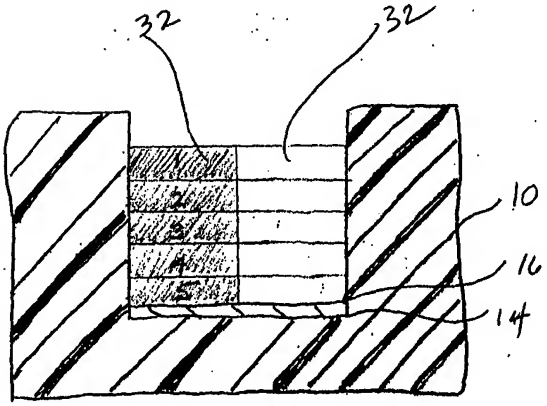
Fig. 8

A.



Case 1: All 10 factors are present
Overall factor concentration = [10/10] = [1]
[1] factor/well

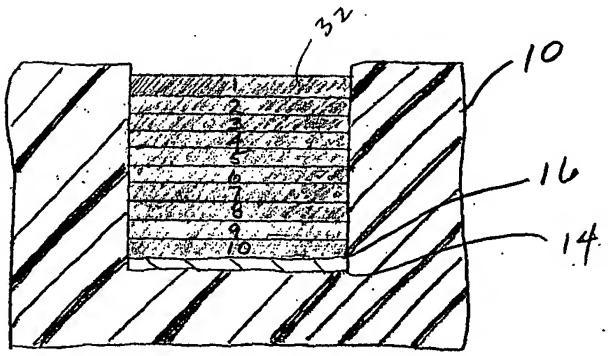
B.



Case 2: 5 out of 10 factors are present
overall factor concentration = [5/10] = [0.5]
[0.5] factor/well

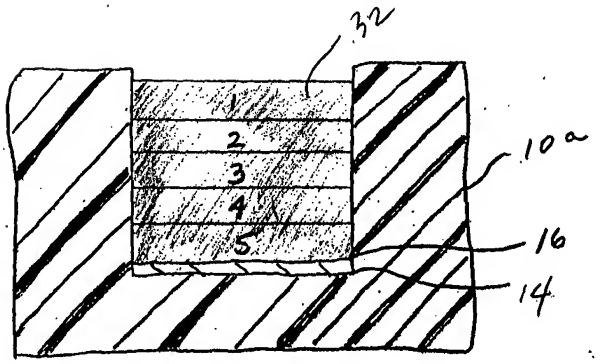
Fig. 9

A.



Case 1 :: All 10 factors are present
Overall factor concentration = [10/10] = [1]
[1] factor/well

B.



Case 2: 5 out of 10 factors are present
overall factor concentration = [1]
[1] factor/well

Run	Type	A:Fibronectin (µl)	B:Collagen I (µl)	C:Vitronectin (µl)	D:Collagen VI (µl)	E:Collagen III (µl)	F:Laminin (µl)	G:Collagen Iv (µl)	H:Elastin (µl)	J:Poly-L-Lysine (µl)	K:Poly-L-Ornithine (µl)
1	CentEdge			25		25					
2	CentEdge	25							25		
3	CentEdge				25				25		
4	Vertex									50	
5	CentEdge				25						25
6	CentEdge	25	25								
7	CentEdge			25						25	
8	CentEdge					25			25		
9	Vertex			50							
10	CentEdge	25					25				
11	Center	5	5	5	5	5	5	5	5	5	5
12	Vertex							50			
13	CentEdge	25						25			
14	CentEdge	25			25						
15	CentEdge	25									25
16	CentEdge		25				25				
17	CentEdge					25	25				
18	Center	5	5	5	5	5	5	5	5	5	5
19	Center	5	5	5	5	5	5	5	5	5	5
20	CentEdge				25		25				
21	CentEdge					25		25			
22	CentEdge		25						25		
23	CentEdge	25				25					
24	CentEdge			25	25						
25	Vertex		50								
26	Vertex	50									
27	CentEdge				25			25			
28	Vertex					50					
29	CentEdge			25						25	
30	CentEdge		25								25
31	Vertex						50				
32	Vertex					50					
33	CentEdge				25					25	
34	CentEdge		25			25					
35	CentEdge				25				25		
36	CentEdge					25				25	
37	CentEdge			25					25		
38	CentEdge			25							25
39	Vertex								50		
40	CentEdge				25	25					
41	Center	5	5	5	5	5	5	5	5	5	5
42	Vertex										50
43	CentEdge	25								25	
44	CentEdge	25		25							
45	CentEdge		25					25			
46	CentEdge		25	25							
47	CentEdge		25							25	
48	Vertex				50						
49	CentEdge			25			25				
50	CentEdge			25				25			
51	CentEdge					25					25
52	CentEdge		25		25						

Figure 10

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		VN / C III	FN / ELA	Col VI / ELA		C VI / PO	FN / CI	VN / PL	C III / ELA		FN / LAM	
C		MID		FN / C IV	FN / C VI	FN / PO	C I / LAM	C III / LAM	MID	MID	C VI / LAM	
D		C III / C IV	C I / ELA	FN / C III	VN / C VI			C VI / C IV		VN / PL	C I / PO	
E			C III	C VI / PL	C I / C III	C III / PL	C III / PL	VN / ELA	VN / PO		C VI / C III	
F		MID		FN / PL	FN / VN	C I / C IV	C I / VN	C I / PL		VN / LAM	VN / C IV	
G		C III / PO	C I / C VI									
H												
			Midpoint - contains all 10 adhesion ligands									
			Single adhesion ligand containing wells									

Figure 11

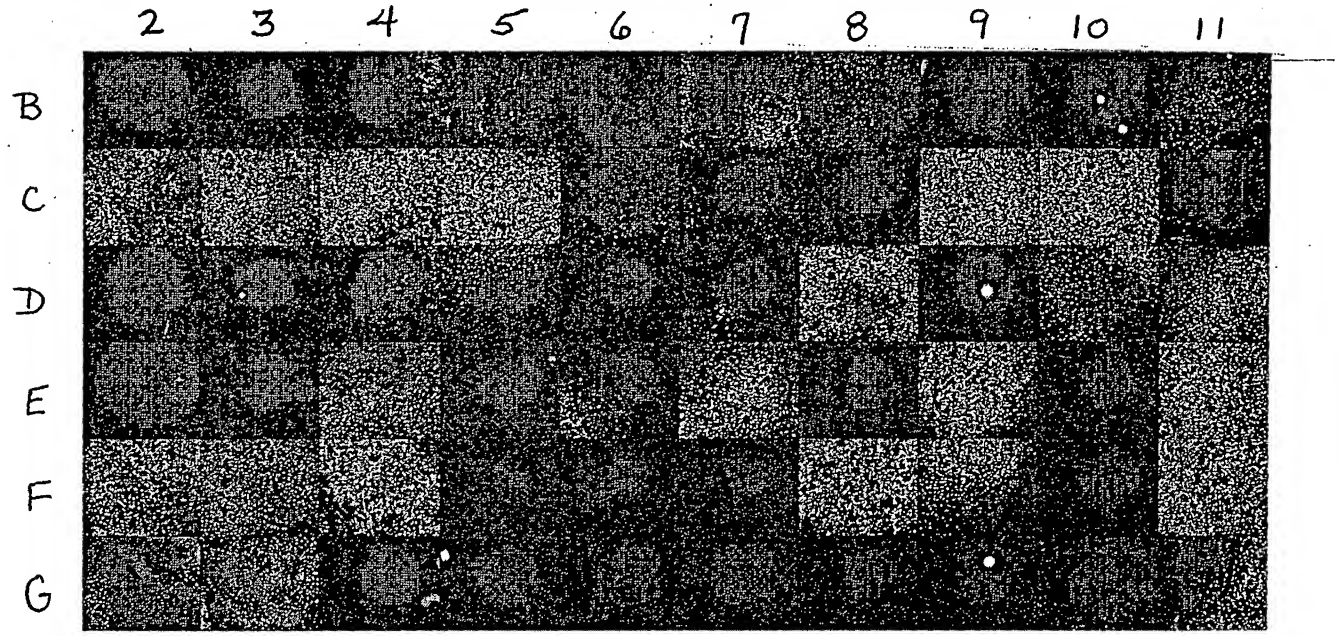


Figure 12

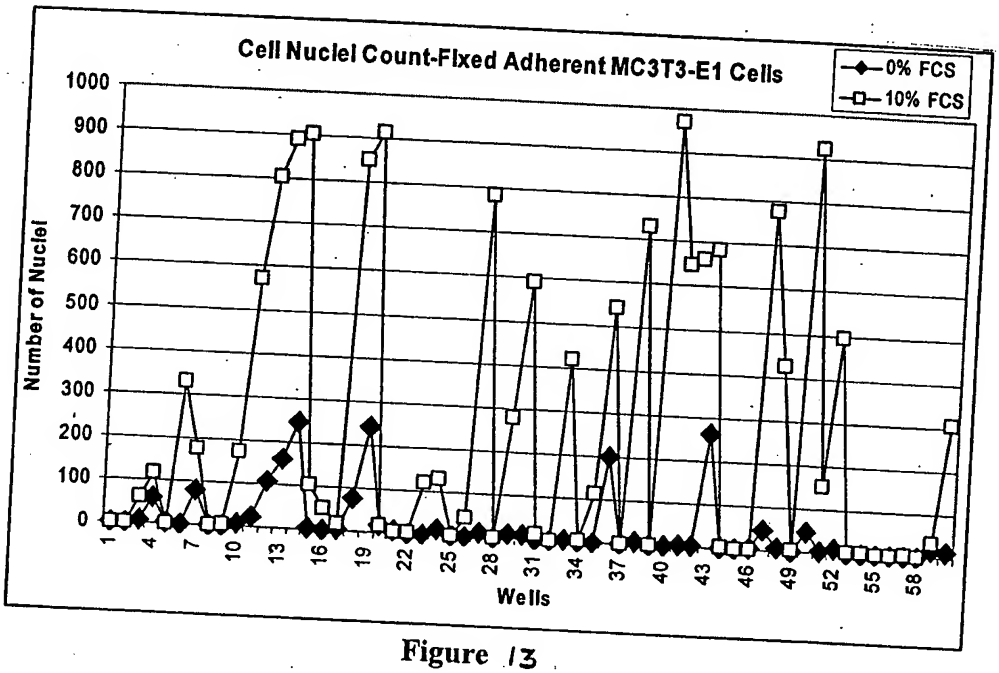
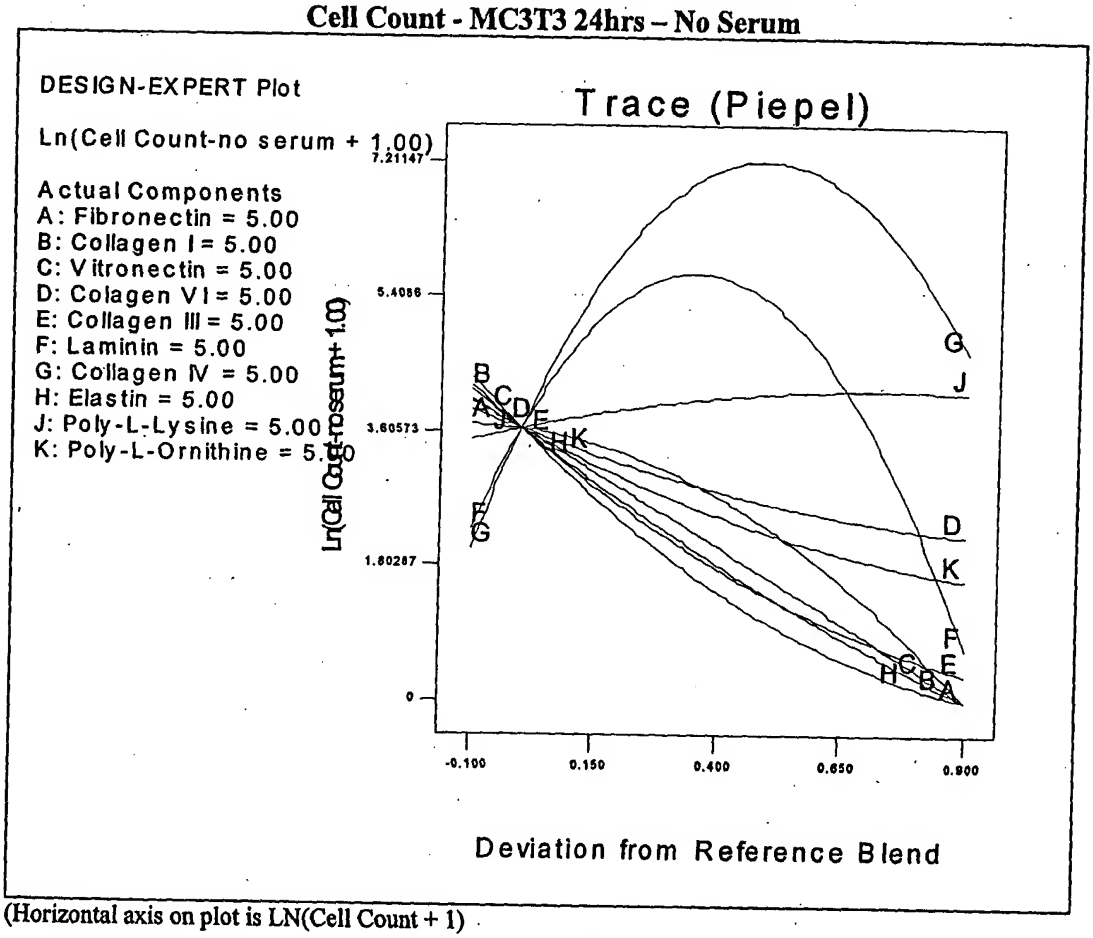

Cell Nuclei Count-Fixed Adherent MC3T3-E1 Cells
0% FCS
10% FCS
Number of Nuclei
1000
900
800
700
600
500
400
300
200
100
0
1
4
7
10
13
16
19
22
25
28
31
34
37
40
43
46
49
52
55
58
Wells

Figure 13

Cell Count - MC3T3 24hrs – No Serum



(Horizontal axis on plot is LN(Cell Count + 1)

Figure 14

Cell Count – MC3T3 24hrs - 10% Serum

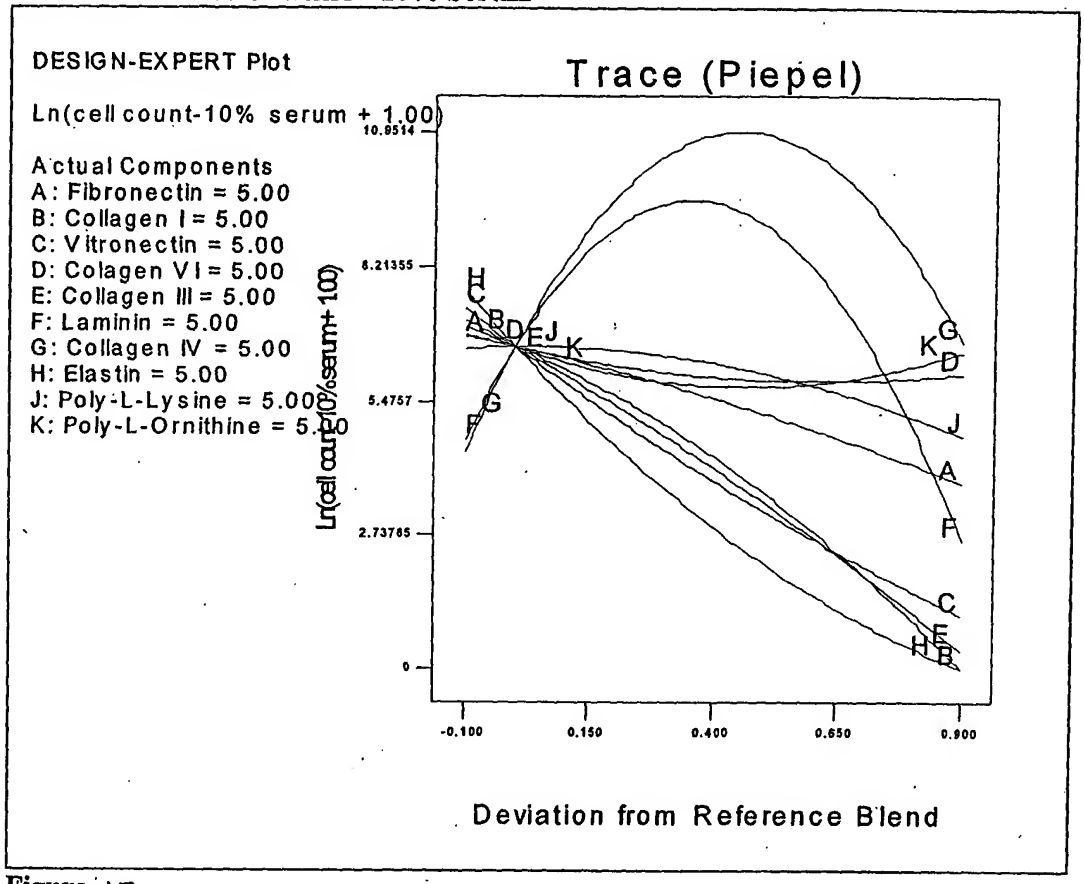


Figure 15

Run	F01	F02	F03	F04	F05	F06	F07	F08
1	-1	-1	-1	1	-1	-1	-1	1
2	-1	-1	1	1	-1	-1	-1	-1
3	1	1	1	-1	-1	-1	-1	-1
4	1	1	1	-1	1	1	1	1
5	1	-1	1	1	1	-1	1	-1
6	-1	-1	-1	1	1	-1	-1	-1
7	1	-1	1	-1	-1	-1	1	-1
8	1	-1	1	-1	1	-1	-1	1
9	-1	1	1	1	1	1	-1	-1
10	1	1	1	1	-1	-1	-1	-1
11	-1	-1	-1	-1	-1	1	1	-1
12	1	-1	-1	-1	-1	-1	1	1
13	1	1	1	-1	-1	1	1	1
14	-1	-1	-1	1	1	-1	1	1
15	1	-1	-1	1	1	1	1	1
16	-1	1	-1	1	-1	-1	-1	1
17	-1	-1	1	-1	-1	-1	1	1
18	1	-1	-1	-1	1	1	-1	1
19	1	1	-1	-1	-1	-1	1	-1
20	1	-1	-1	1	-1	1	1	1
21	-1	-1	-1	-1	1	-1	-1	-1
22	1	-1	1	-1	1	-1	-1	-1
23	-1	-1	1	-1	-1	1	1	1
24	-1	1	-1	-1	1	1	1	-1
25	-1	1	-1	1	-1	-1	1	-1
26	1	1	1	1	1	1	1	1
27	-1	1	1	1	-1	1	1	1
28	-1	1	-1	-1	-1	1	1	-1
29	1	1	-1	1	1	1	1	-1
30	1	-1	-1	1	-1	-1	1	1
31	-1	1	1	-1	1	-1	1	-1
32	1	1	-1	1	-1	1	-1	-1
33	1	1	-1	-1	-1	-1	-1	1
34	1	1	1	1	-1	-1	1	1
35	-1	-1	-1	1	-1	-1	1	-1
36	1	-1	-1	-1	-1	1	-1	-1
37	-1	1	-1	-1	1	-1	1	1
38	1	1	1	1	1	-1	-1	-1
39	1	1	-1	-1	1	1	1	1
40	-1	1	1	1	-1	1	-1	1
41	1	-1	1	1	-1	1	-1	1
42	1	1	1	-1	1	-1	1	-1
43	-1	-1	1	-1	1	1	1	-1
44	-1	1	1	1	1	-1	-1	1
45	-1	1	1	-1	1	1	-1	1
46	1	1	-1	1	1	-1	1	-1
47	1	-1	-1	-1	1	-1	-1	1
48	-1	1	-1	1	1	1	-1	1
49	-1	-1	1	1	1	1	1	-1
50	-1	-1	1	1	-1	1	1	-1
51	-1	-1	1	1	1	-1	1	1
52	1	1	-1	1	-1	1	-1	-1
53	-1	-1	-1	-1	1	1	-1	-1
54	-1	1	-1	-1	-1	1	-1	-1
55	1	-1	-1	1	1	1	-1	1
56	-1	1	-1	-1	1	-1	-1	1
57	-1	-1	1	-1	-1	1	-1	1
58	-1	1	1	-1	-1	-1	-1	1
59	1	-1	1	-1	-1	1	-1	-1
60	1	-1	1	1	1	1	-1	-1

Fig. 16a

F09	F10	F11	F12	F13	F14	F15	F16	F17
1	-1	1	1	-1	1	-1	1	-1
1	-1	-1	-1	1	1	-1	1	1
1	1	-1	-1	-1	-1	1	-1	-1
-1	-1	1	1	1	1	1	-1	-1
1	-1	-1	1	-1	-1	1	1	1
-1	1	-1	-1	-1	1	1	-1	1
-1	1	-1	1	1	1	-1	1	-1
-1	-1	1	1	1	-1	1	1	1
-1	-1	-1	1	1	-1	-1	-1	-1
-1	1	1	-1	-1	-1	-1	1	-1
-1	-1	-1	1	-1	-1	-1	1	1
-1	-1	-1	-1	1	-1	-1	-1	1
1	1	-1	-1	-1	-1	-1	1	1
-1	1	-1	1	-1	-1	-1	1	-1
-1	-1	-1	-1	-1	1	1	-1	-1
-1	-1	1	-1	1	1	1	-1	1
-1	1	1	-1	1	-1	1	-1	-1
1	-1	1	-1	1	-1	-1	-1	1
-1	-1	1	1	-1	1	1	-1	1
-1	1	-1	1	-1	-1	1	-1	-1
1	1	-1	1	1	-1	1	-1	1
1	-1	-1	1	-1	1	1	1	-1
-1	1	1	1	1	-1	-1	1	1
1	1	1	1	-1	-1	1	1	1
-1	1	1	1	-1	1	1	1	1
1	1	1	1	1	1	1	1	1
1	-1	-1	1	1	1	1	1	-1
1	1	-1	1	-1	1	-1	-1	-1
-1	1	1	1	1	1	-1	-1	-1
1	-1	1	1	1	1	-1	-1	1
-1	-1	1	-1	-1	1	-1	1	1
1	-1	-1	1	1	1	-1	1	1
1	-1	-1	-1	-1	1	-1	-1	-1
1	1	1	-1	-1	-1	-1	-1	1
1	1	1	-1	1	-1	1	-1	-1
-1	1	1	-1	1	1	-1	1	-1
1	-1	1	-1	1	-1	-1	1	-1
-1	-1	1	1	-1	-1	-1	-1	1
1	-1	-1	-1	-1	-1	1	1	-1
-1	-1	1	-1	-1	1	1	1	-1
-1	-1	-1	1	-1	-1	1	-1	1
-1	1	-1	-1	1	1	1	-1	1
1	-1	1	-1	-1	1	-1	-1	1
1	1	1	-1	-1	-1	-1	-1	-1
-1	1	-1	1	-1	1	-1	-1	1
1	-1	-1	-1	1	-1	-1	1	-1
-1	1	1	1	-1	1	-1	1	-1
-1	1	-1	-1	1	-1	-1	1	1
-1	-1	-1	-1	1	1	-1	-1	-1
1	-1	1	-1	-1	-1	1	-1	-1
1	1	-1	-1	1	1	1	1	1
-1	1	-1	-1	1	-1	1	1	1
-1	-1	1	-1	-1	-1	1	1	-1
1	-1	1	1	1	-1	1	-1	1
1	1	1	-1	-1	1	1	1	1
1	1	-1	1	1	1	1	-1	-1
1	1	-1	1	-1	1	-1	-1	1
-1	-1	-1	1	1	-1	1	1	-1
1	1	1	-1	1	1	1	1	-1
1	1	1	1	1	-1	-1	-1	-1

Fig. 16b

F18	F19	F20	F21	F22	F23	F24	F25	F26
-1	-1	1	-1	-1	1	-1	1	1
-1	1	-1	1	-1	-1	-1	1	-1
-1	1	1	-1	1	1	-1	1	-1
-1	-1	-1	1	1	-1	-1	-1	-1
-1	1	1	1	1	-1	-1	1	1
1	-1	1	-1	1	-1	-1	-1	1
1	-1	-1	1	-1	-1	1	1	1
1	-1	-1	1	1	1	1	1	-1
-1	-1	-1	-1	1	1	-1	1	1
-1	-1	1	1	-1	1	1	-1	1
-1	1	1	-1	1	-1	1	-1	-1
1	-1	1	1	-1	1	-1	1	-1
-1	-1	-1	-1	1	-1	-1	-1	1
-1	1	-1	1	1	1	-1	1	-1
-1	-1	1	-1	-1	-1	1	1	-1
-1	1	-1	-1	1	-1	-1	1	1
-1	1	-1	-1	1	-1	1	1	1
-1	-1	1	-1	1	1	1	-1	1
-1	1	-1	-1	-1	1	-1	-1	1
1	1	1	-1	1	1	1	1	-1
-1	-1	-1	1	-1	-1	1	-1	1
1	-1	1	-1	-1	1	-1	-1	1
1	1	1	-1	-1	-1	-1	-1	1
1	1	-1	-1	-1	-1	-1	1	1
-1	-1	1	1	1	1	1	-1	-1
1	1	1	1	1	1	1	1	1
-1	-1	-1	-1	1	1	-1	-1	-1
1	-1	-1	1	-1	1	1	1	-1
-1	-1	1	1	-1	-1	-1	-1	1
1	1	1	1	-1	-1	-1	-1	-1
1	-1	1	-1	1	-1	-1	1	-1
1	1	-1	-1	1	1	1	1	1
1	1	-1	1	1	-1	1	-1	1
1	-1	-1	-1	-1	1	-1	-1	-1
1	-1	-1	1	1	1	-1	1	1
1	-1	-1	-1	1	-1	-1	1	-1
-1	1	1	1	-1	1	1	1	1
-1	-1	-1	1	1	-1	1	1	-1
-1	-1	-1	1	-1	-1	-1	1	1
1	1	1	1	-1	-1	1	1	1
1	1	-1	1	-1	1	-1	-1	1
1	1	1	-1	-1	1	1	1	1
1	1	-1	1	1	1	1	-1	-1
1	1	-1	-1	-1	-1	1	-1	-1
-1	1	1	1	-1	1	-1	1	-1
1	1	1	-1	1	-1	1	-1	-1
-1	1	-1	-1	1	1	1	-1	1
1	-1	1	1	1	1	-1	-1	1
-1	1	-1	-1	-1	1	1	-1	1
1	-1	1	1	1	-1	1	-1	1
-1	-1	-1	-1	-1	1	1	-1	-1
-1	1	-1	1	-1	-1	1	-1	-1
1	1	-1	1	-1	1	-1	-1	-1
-1	-1	1	-1	-1	1	1	1	-1
1	-1	-1	-1	-1	-1	1	1	-1
1	1	1	1	1	-1	-1	-1	1
-1	-1	1	1	1	-1	1	-1	1
1	-1	1	-1	-1	-1	1	-1	-1
-1	1	1	1	1	1	-1	-1	-1
-1	1	1	-1	-1	-1	-1	1	-1

Fig. 16c

F27	F28	F29	F30
1	-1	1	-1
-1	1	-1	1
1	-1	-1	-1
1	-1	-1	-1
1	1	1	-1
-1	-1	1	-1
-1	1	1	1
-1	-1	-1	-1
-1	1	-1	1
-1	1	-1	-1
-1	1	-1	-1
-1	-1	1	-1
1	-1	1	1
1	-1	-1	1
1	1	-1	1
1	-1	1	1
-1	1	-1	1
-1	1	-1	-1
-1	1	1	1
-1	1	1	1
1	1	-1	1
1	1	-1	1
1	-1	-1	-1
-1	-1	-1	-1
-1	-1	-1	1
1	1	1	1
-1	1	-1	-1
1	-1	1	-1
-1	-1	-1	1
1	1	-1	-1
-1	1	1	1
-1	-1	-1	-1
-1	-1	-1	1
1	1	-1	1
1	1	-1	-1
1	1	1	-1
-1	-1	1	1
1	-1	1	-1
-1	1	1	-1
1	1	-1	-1
-1	-1	1	1
1	-1	-1	-1
1	1	1	1
-1	1	1	-1
-1	1	-1	-1
1	-1	-1	1
1	1	1	-1
1	1	1	1
1	-1	1	-1
-1	-1	1	-1
-1	-1	1	-1
1	1	1	-1
1	-1	-1	1
1	1	1	1
-1	-1	-1	1
-1	1	1	-1
1	-1	-1	1
1	-1	1	1
-1	-1	1	1
-1	-1	1	1

Fig. 16d

Fig. 17

MPM Factor	Factor	Receptor	Classification
F01	Sonic hedgehog amino-terminal peptide (Shh-N)	PATCHED (PTCH-1) / PTCH-2 / SMO (smoothened)	7-pass transmembrane / 7-pass transmembrane / GPCR
F02	BMP-cocktail	BMPRc-1A, BMPRc-1B, BMPRc-2	BMPR-Ser/Thr Kinase
F03	Cholesterol (water soluble formulation)	LDL Rc / SR-BI	channels & membrane transporters
F04	Leptin (human, recombinant)	Leptin Receptor	Cytokine Rc
F05	Prolactin (human, recombinant)	Prolactin Receptor	Cytokine Rc
F06	Ciliary neurotrophic factor (CNTF) (human, recombinant)	CNTFR-alpha + gp130 + LIF Rc	Cytokine Rc
F07	Amphiregulin (long form, recombinant)	EGFR	EGFR-tyrosine kinase
F08	Fibroblast Growth Factor-8c (FGF-8c) (mouse, recombinant)	FGF Rc Family	FGFR-tyrosine kinase
F09	Fibroblast Growth Factor-7 (FGF-7) = KGF	FGF Rc Family	FGFR-tyrosine kinase
F10	Vasoactive Intestinal Peptide (VIP)	VPAC1R / VPAC2R	GPCR
F11	Gastrin/CCK8-cocktail	CCK-B/Gastrin Rc	GPCR
F12	Neuropeptide Y	Neuropeptide Y Rc Family (Y1-Y6)	GPCR
F13	Thrombin/TXA2-cocktail	thromboxane A2 Receptor	GPCR
F14	C natriuretic peptide) (human, porcine, rat frag 32-53)(CNP)	Guanylate Cyclase B (GC-B) Rc (ANPR-A & ANPR-B)	Guanylyl Cyclase
F15	Interleukin-3 (IL-3) (human, recombinant)	IL3Rc-beta (aka GMCSFRc) / IL3Rc-alpha	IL-Cytokine Rc
F16	Interleukin-18 (IL-18) (human, recombinant)	IL18Rc	IL-Cytokine Rc
F17	Midkine (MK) (human, recombinant)	PTPzeta	Miscellaneous
F18	Neurturin (NTN)	GFRa1 / GFRa2 / c-ret	Miscellaneous
F19	Dibutyryl cyclic AMP	cAMP Receptor Protein Kinase (PKA)	Ser/Thr Kinase
F20	DMF (n n dimethylformamide); a polar solvent	Not receptor mediated	Small Molecule
F21	Cycloheximide (actidione)	Not receptor mediated	Small Molecule
F22	Platelet-derived endothelial cell growth factor (PD-ECGF) (aka thymidine phosphorylase)	Not Receptor mediated	Small Molecule
F23	Laminin	Laminin-Elastin Rc / alpha6 beta4 integrin	surface-matrix receptor
F24	Transforming Growth Factor beta3 (human, recombinant)	TGFBRc-1, TGFBRc-2, TGFBRc-5	TGFBR-Ser/Thr Kinase
F25	Estradiol, beta (water soluble formulation)	Estrogen Receptor-alpha (ER-A) / Estrogen Receptor-beta (ER-B) / Estrogen-related receptor alpha (ERR-A) / Estrogen-related Receptor beta (ERR-B)	Transcription Factor
F26	Hydrocortisone	Hydrocortisone Rc	Transcription Factor
F27	nuclear factor of activated T cells (NFAT) proteins (NFAT1-NFAT5)	Not Receptor mediated	Transcription Factor
F28	Hepatocyte Growth Factor (HGF, scatter factor)	c-Met (HGFR)	tyrosine kinase
F29	Growth Hormone	GH Receptor	tyrosine kinase
F30	Brain-derived Neurotrophic Factor (BDNF) (human, recombinant)	TrkB	tyrosine kinase